IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Mark Madden et al. Art Unit : 1652
Serial No. : 09/751,299 Examiner : Kathleen M. Kerr
Filed : December 28, 2000
Title : METHODS FOR PRODUCING ALPHA-SUBSTITUTED CARBOXYLIC ACIDS (amended)

#18
B.g.J
7/28/03

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I, Jennifer Ann Chaplin, am a co-inventor with Mark Madden and David Weiner, on the above-identified patent application.

2. I am an expert in the field of biochemistry and was an expert at the time of the invention. I am presently employed as a research scientist at Diversa Corporation, San Diego, CA, assignee of the above-referenced patent application. My resume is attached as documentation of my credentials.

3. In reviewing the specification for preparing for this declaration, it was noticed that an inadvertent error was made in the protocol as set forth in Example 1 on page 76 (78) of the specification. The cell lysates contained nitrilase which had been overexpressed in an *E. coli* host, not a *Pseudomonas* host (see line 4 of page 76 (78)).

4. I declare that using the teaching of the specification, including the exemplary protocol as set forth in Example 1, pages 77 and 78, of specification, and other protocols known in the art at the time of the invention, alternative protocols, including the protocol described

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I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

7/17/03
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Jeanne Amour Rice
Signature

Jeanne Amour Rice
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below, could have been designed by one skilled in the art at the time of the invention to successfully practice the methods of the invention, i.e., to produce an enantiomerically pure α-substituted carboxylic acid by contacting an aldehyde or ketone with a cyanide-containing compound and an ammonia-containing compound or an ammonium salt or an amine, and stereoselectively hydrolyzing a resulting amino nitrile or cyanohydrin intermediate with a recombinantly generated nitrilase or polypeptide having a nitrilase activity, where the nitrilase is sufficiently active to perform the hydrolysis in the presence of the reaction components, under conditions and for a time sufficient to produce the enantiomerically pure α-substituted carboxylic acid.

5. I declare that at the time of the invention, with the teaching of the specification, it would have taken only routine screening by one skilled in the art to identify recombinant nitrilase enzymes capable of producing an enantiomerically pure alpha-substituted carboxylic acid by combining an aldehyde or ketone with a cyanide and ammonia or an ammonium salt or an amine.

6. I declare that an enantiomerically pure (S)-phenylglycine was ~~producing~~ produced using the same nitrilase gene as BD1911, except that the gene was overexpressed in *Psuedomonas* instead of *E. coli*. The protocol for producing the enantiomerically pure (S)-phenylglycine was as follows: 3.15 g of crude phenylglycinonitrile (free base) was dissolved in 53.2 ml methanol. BD 1911, 282ni1, was dissolved in 3.33 ml dH2O; the final concentration was 6 mg protein/ml. KCN stock: 162.8 mg KCN in 10 ml of $NaHCO_3$ buffer (0.1 M; pH 10.6). 0.5 ml of phenylglycinonitrile stock was added to 7.5 ml $NaHCO_3$ buffer (0.1 M, pH 10.2). To this mixture was added 1 ml of the enzyme stock and 1 ml of the KCN stock. The reaction was run on a Metrohm pH-stat with the pH controlled at 10.6 with 0.25 M NaOH. The reaction was run under these conditions for 23 hours. A yield of 58.8% and ee for (S)-phenylglycine of 91.3% was measured by HPLC: Crownpak CR (+) column 4 x 150 mm, 5 um from Chiral Technologies; mobile phase consisting of 1% MeOH, 40% 0.1 M perchloric acid, 59% dH_2O; flow rate 1 ml/min, 25°C, 210 nm.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted

Date: 7/16/03

JChaplin

Jennifer Ann Chaplin

Jennifer A. Chaplin

Jennifer A. Chaplin joined Diversa in July 2000 and is currently a Staff Scientist in the Integrated Chemical Products group. During that time, she has been involved in a number of projects, focusing primarily on the identification and optimization of enzymes for the production of commercially relevant molecules. She played a leading role in the development of the Diversa's nitrilase platform, where she was the project leader for a customer collaboration which involved the discovery and identification of key nitrilases for producing chiral intermediates, more particularly α-carboxylic acids. Ms Chaplin and her team were responsible for the screening and characterization of the nitrilases under process-relevant conditions. More recently, her group has been involved in the identification and optimization of an esterase for an important step in the production of a key pharmaceutical intermediate. In addition, she has contributed to assay development in projects involving oxidative enzymes, such as peroxidases and monooxygenase.

Prior to joining Diversa, Ms Chaplin spent just over one year at Albany Molecular Research, Inc. (formerly EnzyMed, Inc.), where she focused on oxidative reactions involved in combinatorial biocatalysis. She also spent 10 years with AECI (now CSIR Bio/Chemical Technologies, South Africa) where she gained experience in the use of a variety of enzymes under aqueous and non-aqueous conditions. She was responsible for the technical direction of a project involving the chiral synthesis of a flavor compound, which involved a multidisciplinary group of scientists and engineers.

During her career, Ms Chaplin has contributed to patents, peer-reviewed publications and numerous internal publications. She has also been involved in customer interactions and presentations and is actively pursuing SBIR grant opportunities.

Curriculum Vita

Diversa Corporation, July 2000-present
Staff Scientist

EnzyMed, a Division of Albany Molecular Research, Inc.1999-July 2000
Scientist

AECI R&DD/CSIR Department of Food and Biochemical Technology, South Africa, 1989-1999
Senior Research Scientist

Education

B.Sc. University of the Witwatersrand, South Africa, 1988
B.Sc. (Hons.) University of the Witwatersrand, South Africa, 1989

Publications/patents

De Santis, G. Chaplin, J.A. Chi, E. Milan, A. Short, J.M. Weiner, D. Burk, M.J. Mathur, E.J. Madden, M. 2003. Bacterial nitrilase and gene sequences exhibiting stereoselectivity useful for synthesis of chiral reaction products. PCT Int. Appl. WO 0300840

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Brebner, D.K. Chaplin, J.A. Herrera, V.E. MacDonald, M.Y. Skeef, N.S. Worrall, E.D. 1994. Patent no AU 9338539, ZA 9302792, NZ 247610. *Bacillus thuringiensis* insecticide composition